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Antibody Studies in a Patient With Acute Thrombocytopenia Following Infusion of Plasma Containing Anti-PI^{A1}

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Immune thrombocytopenia due to passive transfer of anti-PI^{A1} alloantibody has been noted as a rare but potentially dangerous complication of plasma transfusions. We report a patient with a preoperative platelet count of 241 × 10^9/l who developed severe thrombocytopenia within 2 hr following transfusion of 2 U of fresh frozen plasma. The plasma donor was found to be a PI^{A1}-negative woman. The platelet count of the PI^{A1}-positive patient recovered within 7 days to normal values. In the frozen plasma, excessive antibody binding to GPIIb-IIIa on the recipient's platelets was detected. The antibody was shown to have anti-PI^{A1}-specificity. Only 40 min after transfusion of the frozen plasma, no antibody was detected in the plasma of the recipient. This case suggests that passively administered anti-PI^{A1} alloantibody is immediately adsorbed onto the recipient's platelets and thus removed from circulation. Am. J. Hematol. 56:119–121, 1997.

Key words: purpura; thrombocytopenic; blood transfusion; isoantibodies; platelet membrane glycoproteins

INTRODUCTION

Alloimmune thrombocytopenias, such as neonatal thrombocytopenia and posttransfusion purpura, are due to alloantibodies directed in the majority of these patients against the PI^{A1} antigen located on platelet glycoprotein (GP) IIIa [1,2]. It is widely believed that binding of these alloantibodies to the platelet membrane results in an increased platelet destruction by macrophages in the reticuloendothelial system (RES) [3]. Although numerous studies support this hypothesis, the exact relation between antibody binding and platelet removal in the RES is poorly understood. Strong evidence for a direct cause-and-effect relation has been presented by 2 studies demonstrating that accidental infusion of anti-PI^{A1} alloantibody into a PI^{A1} positive recipient produced severe thrombocytopenia [4–6]. Unfortunately, the authors were unable to evaluate the course of the alloantibody in the recipients.

In this study, we report a case of severe immune thrombocytopenia in a PI^{A1} positive patient caused by infusion of anti-PI^{A1} containing fresh frozen plasma. Evaluation of the alloantibody in the recipient suggested that immediately after infusion, all alloantibody was adsorbed onto the recipient's platelets.

CASE REPORT

The patient was a 55-year-old male diagnosed with terminal renal insufficiency who was admitted to the hospital for renal transplantation. Since the patient had experienced repeated episodes of thrombotic occlusion of his dialysis fistula, he had been treated with warfarin since one year. On admission, his Hb was 13.5 g/L, WBC 5.9 × 10^9/L, platelets 241 × 10^9/L, and prothrombin time

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(PT) 30%. Because of the planned surgery, the patient was transfused with 2 U of fresh frozen plasma (FFP) over 1 hr and given vitamin K intravenously for correction of his coagulation. Forty minutes following plasma infusion, PT was 60% and 2 hr following FFP infusion, platelets had fallen to 1×10^9/L. The patient developed mucosal bruising but no major bleeding. The surgery was postponed and he was given high dose intravenous immunoglobulins (IvIgG, 30 g per day) over 5 days. A bone marrow examination revealed a normocellular marrow with normal megacaryopoiesis. The subsequent clinical course was without further events and the patient's platelet count returned to normal within the following 8 days. The donor of the fresh frozen plasma, a 49-year-old female was recalled. Platelet typing and platelet antibody assays were performed in both patient and donor.

IN VITRO STUDIES

Antibody Studies

EDTA-anticoagulated blood was collected from the recipient and all members of the donor's family. The course of the alloantibody and the platelet count in the recipient was followed until normalization.

The immunobead assay was performed with minor modifications as described in detail elsewhere [7]. To evaluate platelet-bound antibodies, donor, recipient, or normal platelets were separated by differential centrifugation. For measurement of plasma autoantibodies, washed normal platelets (10^8) were incubated with either donor, recipient, or normal control plasma. Immunobeads were prepared by incubating 1/4-inch polystyrene beads with murine monoclonal antibody (MoAb) against GPIIb-IIIa (2A9; provided by Dr. Virgil Woods, University of California, San Diego). For assay, donor or recipient platelets (10^8) or control platelets from a healthy blood donor sensitized with donor or recipient plasma were solubilized in 1% Triton X-100 (BDH Chemicals Ltd, Poole, England). The lysate was then incubated for 60 min with immunobeads coated with anti-GPIIb-IIIa. After washing, any immunobead-bound autoantibody-GPIIb-IIIa complexes were detected by radiolabeled monoclonal anti-human IgG (HB 43). Results are expressed as a binding ratio (BR) of the donor/recipient plasma (cpm) divided by the mean cpm of at least three control samples. Donor/recipient samples with a BR > 1.4 (>mean of controls + 3 SD) are considered positive.

Platelet Typing and Serologic Characterization of the Alloantibody

Platelets were typed using anti-PI^{A1} and anti-PI^{A2} serum [8]. The donor's alloantibody was evaluated using PI^{A1}/PI^{A2} positive and PI^{A1}/PI^{A2} negative platelets [9].

TABLE I. Anti-GPIIb-IIIa Antibody Binding to Platelets (Plt) From Various Donors*

	Donor plasma	Recipient $plasma_{(pre)}$[a]	Recipient $plasma_{(post)}$[a]
Plt donor	1.0	—	—
Plt recipient	155.9	0.7	1.3
Control Plt (PI^{A1}-pos.)	199.6	—	—

*Results are expressed as anti-GPIIb-IIIa antibody binding ratio (positive >1.4).

[a]Plasma of recipient drawn before (pre) and 40 min following (post) transfusion of FFP.

RESULTS AND DISCUSSION

The PI^{A1} antigen is present on platelet GPIIIa of 97% of the population. It is responsible for 50–75% of the cases of fetal or neonatal alloimmunothrombocytopenia as well as for more than 90% of the patients with posttransfusion purpura [2]. Posttransfusion purpura due to passive transfer of anti-PI^{A1} has been reported 6–12 hr following transfusion [4,5]. Our patient developed severe thrombocytopenia within 2 hr following transfusion of 500 ml fresh frozen plasma. Analysis of the donor plasma revealed anti-GPIIb-IIIa antibody in her plasma reacting with recipient as well as with random platelets but not with platelets obtained from the same donor (Table I). Further evaluation of donor plasma showed that the antibody reacted specifically with PI^{A1} positive platelets, but failed to bind to PI^{A1} negative platelets (Table I). Typing of the recipients platelets revealed PI^{A1}-positivity, whereas the platelets of the donor were PI^{A1} negative.

It is noteworthy that only 40 min following transfusion of the FFP, no alloantibody could be detected in the recipient plasma (Table I). In contrast, dilution experiments using different ratios of donor and recipient plasma revealed detectable alloantibody at a ratio of up to 1:200 (Fig. 1). Since 500 ml of transfused plasma into a recipient of 71 kg would approximately be diluted by 1:7, these results show that only 40 min after infusion, >99.5% of alloantibody was adsorbed onto recipient platelets and therefore removed. These findings are of therapeutical importance and suggest that any therapy aiming for removal of the alloantibody or for inhibition of the destruction of antibody-coated platelets by the RES should have been started immediately after transfusion, i.e., within 40 min in this case. Therefore, in retrospect, the rational for treatment of our patient with IvIgG over 5 days was poor for increasing the platelet count. We, therefore, suggest that patients with severe and symptomatic thrombocytopenia due to passive transfer of alloantibodies rather be treated by immediate platelet transfusions.

The donor was a 49-year-old female with a history of 5 pregnancies, which, all except one PI^{A1}-negative son,

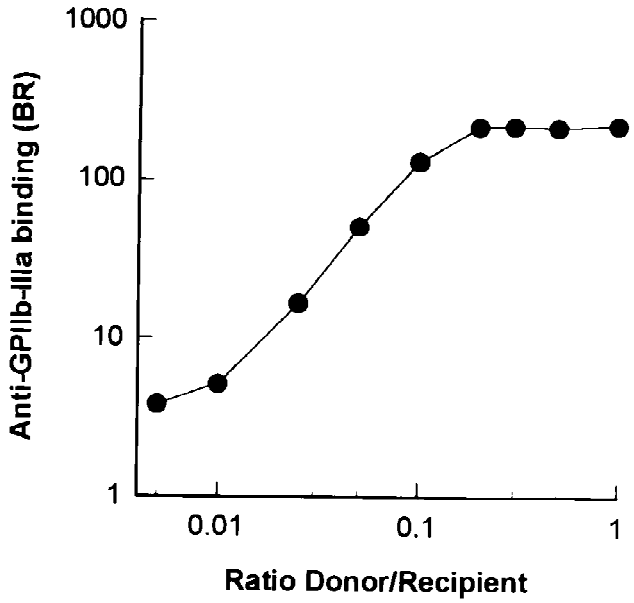

Fig. 1. Dilution experiments using different ratios of donor and recipient plasma revealed detectable alloantibody at a ratio of up to 1:200.

ended in miscarriages. At an age of 24 years she received a single blood transfusion at the time of her second miscarriage. Within the last 13 years, she donated blood 13 times. We were able to identify another incident where plasma obtained from one of her blood donations induced severe thrombocytopenia in a female patient who underwent craniotomy. Similar to our case, platelet count fell from normal (284×10^9/L) to 6×10^9/L within 4 h in this patient. However, after immediate treatment with platelet transfusions, her platelet count was 50×10^9/L on the next day and recovered to normal within 5 days. This further strengthens the suggestion that infused alloantibody is immediately adsorbed and removed by the autologous platelets and that transfusion of random platelets is an effective treatment.

In summary, thrombocytopenia due to passive transfer of anti-PI^{A1} alloantibody represents a rare but potentially dangerous complication of plasma transfusions. Since the alloantibody may be adsorbed onto the patient's platelets immediately after infusion, platelet transfusions are considered the most effective treatment.

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